In summary, the Examiner's position appears to be that:

A. Ekins '031 exemplifies the use of small amounts of binding agent which allegedly is as low as 0.25V/K moles;

B. There is no disclosure in Ekins '031 to teach the person of ordinary skill in the art that 0.25V/K moles of binding agent represents the lower limit at which it is possible to carry out the disclosed assay;

C. Hence, the barrier to arriving at the present invention was practical, and not theoretical, and at the priority date of the above application it would have been possible to solve these practical problems in an obvious way.

Applicant respectfully takes exception to the Examiner's conclusion inasmuch as the Examiner impermissibly uses hindsight to interpret the prior art in a manner in which the person of ordinary skill in the art would not have done at the priority date. In addition, the Examiner's position ignores the contribution that the claimed invention, provides, as a whole, as compared to the prior art.

It has long been recognized that certain tenets of patent law must be taken into account when applying 35 U.S.C. §103 in a particular case. These include: (i) the claimed invention must be considered as a whole, In Re Antonie, 195 U.S.P.Q. 6 (CCPA 1977); (ii) the prior art references must be considered in their entireties as they would be by those skilled in the art and the references must suggest the desirability and thus the obviousness of making the proposed substitution, combination or other modification, In Re Rinehart, 189 U.S.P.Q. 143 (CCPA 1976); In Re Lintner, 173 U.S.P.Q. 560 (CCPA 1972); and (iii) the prior art references must be viewed without the benefit of hindsight afforded by knowledge of the Applicant's invention. Ex parte Stauber 208

U.S.P.Q. 945 (Bd. Apps. 1980). In the present case, the Examiner has clearly contravened these tenets in concluding that Applicant's assay would have been obvious to those of ordinary skill in the art at the time the invention was made in view of the disclosures of Ekins '031 and Leaback.

A. THE PRIOR ART FAILS TO SUGGEST THE ADVANTAGE OF USING LESS THAN 0.1V/K MOLES OF BINDING AGENT TO ASSAY DESIGN

The prior art fails to suggest any advantage resulting from using less than 0.1V/K moles of binding agent to assay design.

As noted by the Court in In Re Antonie, supra at 8,

> *In delineating the invention as a whole, we look not only to the subject matter which is literally recited in the claim in question . . . but also to those properties of the subject matter which are inherent in the subject matter and are disclosed in the specification. [Emphasis in original]*

Thus, in the present case, the invention, as a whole, includes the surprising result that it is possible to substantially reduce the amount of binding agent used relative to Ekins '031, and yet the resulting assay has equivalent or superior sensitivity. This distinct benefit is not even remotely suggested in the prior art of record. The advantage of using less than 0.1V/K moles of binding agent to assay design is summarized in FIG. 1 of the present application. FIG. 1 demonstrates that as [Ab] falls, the fractional occupancy of Ab sites by An reaches an essentially constant level dependent on [An].

The flat portions of the curves where this effect is observed are below about 0.1V/K moles of Ab. This is why the 0.1V/K ratio is a feature of all the independent claims of the application.

Thus, by using an amount of binding agent less than about 0.1V/K moles, the person carrying out the assay

automatically ensures that they are operating on the flat part of any of the curves where F is dependent on [An]. This means that a small amount of analyte is removed from the sample irrespective of the total analyte concentration.

There is no teaching or suggestion in the prior art to carry out such an analysis, and so the person of ordinary skill in the art would not have arrived at the theoretical conclusion that underpins the present invention.

This result is clearly surprising as even today those skilled in the art believe that such small amounts of binding agent would inevitably be swamped by analyte and fail to provide a workable assay. See for example paragraph 6 of the Roitt declaration.

II. THE EXAMINER HAS NOT CITED ANY PRIOR ART THAT DISCLOSES THE REQUIREMENT OF USING LESS THAN 0.1V/K MOLES OF BINDING AGENT

It is especially noteworthy that the cited prior art does not disclose anywhere the requirement of using a plurality of spots each containing less than 0.1V/K moles of binding agent, much less that the use of such a small amount of binding agent allows the determination of a plurality of analyte concentrations in a reliable and sensitive way.

The prior art does not disclose these features of the invention which are set forth in the claims and which lead to these surprising and unexpected advantages.

Although Ekins '031 does disclose an example of an assay which uses about 0.25V/K moles of binding agent, Ekins '031 does not disclose that it is either theoretically or practically possible to reduce this amount and produce a sensitive assay. Indeed, as noted on page 1 of the instant application and noted by the Examiner, Ekins '031 is solely concerned with the matter of when assays become sample-volume independent.

Thus, at the priority date of this application, Ekins '031 provides no teaching to the person of ordinary

skill in the art:

A. To <u>further</u> reduce the amount of binding agent;

B. That an assay can be <u>just as sensitive</u> using such a small amount of binding agent, as Ekins '031 does not even deal with this issue; or

C. Of the practical advantages of only needing to know the volume and affinity constant to use the assay, instead of the necessity of knowing the <u>expected concentration</u> required by the assay of Ekins '031.

These unobvious advantages were extensively discussed in the response to the last Office Action and the averments in the Roitt and Berger declarations.

Given the prevailing view in the art that such small amounts of binding agent would inevitably lead to the binding agent being swamped by the far greater amounts of analyte in the sample, it is respectfully submitted that it is surprising that it is possible to reduce the amount of binding agent in comparison to that used in Ekins '031 and retain or enhance sensitivity.

As an example of this view in the art, the Examiner's attention is again directed to the view expressed in the 1991 article by the American Thyroid Association filed with the last response, which on page 2006 recommends that in order to optimize sensitivity, the practitioner should use an amount of binding agent to "bind the majority of analyte in the sample".

The Examiner is also referred to the cited 1993 Clinical Chemistry article, paragraph 5 of the Berger declaration and paragraph 6 of the Roitt declaration. This evidence clearly shows that assays according to the present invention can be many times more sensitive than prior art assays for the same analytee, a fact that even today people skilled in the art find astonishing.

It is settled law that silence in a reference is not a proper substitute for an adequate disclosure of facts from which a conclusion of obviousness may justifiably follow. <u>In</u>

Re Burt, 148 U.S.P.Q. 548 (CCPA 1966). In the present case, Ekins '031 is silent with regard to the use of less than 0.1V/K moles of binding agent in the assay there described and the attendant advantages from operating the assay in such a way. The Examiner, for his part, attempts to bolster the manifestly inadequate disclosure of facts in Ekins '031 on the basis of unsupported assumptions as to the manner in which those skilled in the art would interpret the disclosure of Ekins '031. Such assumptions, however, are clearly refuted by the previously submitted Berger declaration and Roitt declaration. See, especially paragraph 5 of the Berger declaration and paragraph 6 of the Roitt declaration. These two declarations are consistent with the disclosure in Clinical Chemistry 37:2002-2008 (1991) (recommendation of American Thyroid Association regarding optimization of sensitivity for TSH assays), which is of record herein.

Further evidence regarding the manner in which the disclosure of Ekins '031 would be interpreted by those of ordinary skill in the art is provided by the declaration of Professor Roger Philip Ekins which is submitted herewith. The gist of Professor Ekins' declaration is set forth in paragraph 10, which states:

> *In summary, my earlier invention in Ekins '031 makes no mention of assay sensitivity, nor does it give any indication that assays of greater sensitivity (and requiring shorter incubation times) than those of classic design could be achieved. It therefore provides not teaching to the person of ordinary skill in the art in the direction of the present invention, which yields assays of greater sensitivity requiring times of incubation as short as, or shorter than, assays of conventional design, in total contradiction to all accepted ideas in the field.*

Arrayed against the considerable evidence of non-obviousness on the record in this application, the Examiner's contrary finding is plainly untenable. As noted by the PTO

Board of Appeals in Ex parte Wolters, 214 U.S.P.Q. 735 (Bd. Apps. 1979), the Examiner's burden of establishing a *prima facie* case of obviousness is not met by assuming the presence of claim requirements that are missing from the prior art.

III. THOSE SKILLED IN THE ART WOULD NOT HAVE EXPECTED TO OBTAIN AN ASSAY WITH ACCEPTABLE KINETICS USING 0.1V/K MOLES OF BINDING AGENT

One of the reasons behind the recommendation of the American Thyroid Association to use high concentrations of binding agent (e.g. antibody) is the widely held view that this causes the binding reaction between binding agent and analyte to proceed at a faster rate and reach equilibrium sooner. Clearly, the provision of faster assays is a desirable goal to those skilled in the art. The reason for this expectation can be seen from a consideration of the mass action laws:

Rate of formulation of bound antigen complex=k_a[BA][An]

Where k_a= the association rate constant, and [BA] and [An] are the binding agent and analyte concentrations, respectively.

Thus, the greater the concentration of the binding agent, the greater the proportion of the analyte present in the system which is bound in any given time interval. The corollary of the equation is that the reaction rate is reduced, and the time to reach equilibrium is greatly prolonged, by the use of low concentrations of binding agent. This implies that long incubation times are required to allow the analyte molecules to find and bind to binding agent immobilized on a surface.

In other words, the use of high concentrations of binding agent is generally seen as implying that desired sensitivities are achieved in shorter incubation times. As a principal direction of research is to reduce the time it takes to carry out assays, especially in the context of rapid automatic immunoanalyzers, it is respectfully submitted that

the Examiner is mistaken to say that reducing the amount of binding agent would have been obvious, as this runs contrary to generally accepted ideas in the field.

Contrary to these expectations, the practice of the present invention, using less than 0.1V/K moles of binding agent immobilized as microspots, surprisingly provides assays that yield equivalent or improved assay sensitivities in the same or shorter incubation times.

IV. SENSITIVITY IS AN IMPORTANT PROPERTY OF THE ASSAY OF THE PRESENT INVENTION REALIZED BY THE USE OF SPOTS CONTAINING LESS THAN 0.1V/K MOLES OF BINDING AGENT

A. View in the Prior Art Concerning Maximizing Sensitivity

Before the present invention, there was a strong prejudice against reducing the amount of binding agent used in assays, the belief amongst those skilled in the art being that this would inevitably have a detrimental effect on sensitivity (see in this context the work of Berson and Yalow).

B. Ekins '031 Does Not Deal With the Issue of Sensitivity

Ekins '031 does not deal specifically with the issue of sensitivity, but with the different problem of assays that are sample-volume dependent. Thus, Ekins '031 provides a way of measuring analyte concentrations where the sample volume does not need to be known. This in itself might be useful in the taking of *in vivo* measurements, e.g. of hormone concentrations in saliva or blood, but does not teach the person of ordinary skill in the art anything about assay sensitivity.

As Ekins '031 does not operate under the restriction of using less than 0.1V/K moles of binding agent, it cannot teach the sensitivity advantages this provides to the person of ordinary skill in the art. The Examiner's attention is respectfully directed to page 4, lines 24-27 of the present application which states:

The present invention involves

the realization that the use of high quantities of binding agent is neither necessary for good sensitivity in immunoassays nor is it generally desirable.

This passage really encapsulates what the present invention is all about; that is the present invention represents a teaching that goes off at a complete tangent from the prior art, including Ekins '031.

C. The Examiner's Argument Relies Upon Hindsight in Interpreting the Teaching Provided by Ekins '031

While it is true that Ekins '031 uses a small amount of binding agent, that with hindsight can be seen to come closer to the limit given in this application than any of the other prior art, this is done for a completely different reason in Ekins '031. Accordingly, before this invention was made, the skilled person would not have realized its significance from the disclosure of Ekins '031.

It is surely only with hindsight knowledge of the present invention that the Examiner made the calculation to determine the amount of binding agent used in Ekins '031 in terms of V and K. It is respectfully submitted that the person of ordinary skill and perception at the priority date would not have made such a calculation; still less would he have seen any significance in it which would lead him in the direction of the present invention. At that time, practitioners in the field were not looking to further reduce the amount of binding agent used in assays, but rather looking to use large amounts of binding agent which was thought to maximize sensitivity and optimize reaction speed.

In summary, it is respectfully submitted that as Ekins '031 does not suggest the use of less than 0.1V/K moles of binding agent or the beneficial impact of this on assay design, it is not really appropriate to infer that the person of ordinary skill in the art would, at the priority date, have derived from Ekins '031 that which is only apparent looking

back from the perspective of the present invention.

The Examiner also argues that it was obvious that sensitivity could be improved by taking lower values of V/K "if one were able to shrink the area to which antibody was applied", provided that the "observation window" was also reduced in size to reduce signal-to-noise. However, the cited prior art does not indicate any such perception by the skilled person at the time the present invention was made.

The Examiner maintains that the motivation for this would come from the skilled person being unable to detect small amounts of the binding agent immobilized over a large area, and going on to shrink the spot size to obtain a workable assay. However, where in the prior art is the problem considered? If such a problem had been perceived by the skilled person, surely the kind of action suggested by the Examiner would have been documented long ago.

The Examiner will appreciate that the mind set of the ordinarily skilled person at the time the present invention was made was influenced by teachings which look in the opposite direction to that suggested by the Examiner. For example, reducing the amount of binding agent would be going into uncharted territory, where it was thought not possible to obtain assays, let alone sensitive and rapid assays; and it clearly had not been realized that reducing the area over which the binding agent is immobilized could improve sensitivity.

In fact, the sensitivity of an assay depends on a range of factors that are not well understood even today. In the case of microspots as claimed in the present invention, conflicting factors affect the sensitivity obtained.

V. THE BARRIER TO THE PRESENT INVENTION WAS THEORETICAL AS WELL AS PRACTICAL

A main theme of the Examiner's argument on pages 3 and 4 of the Office Action, is that practical considerations were the only barrier to using the reduced amounts of binding agent of the present invention.

With the greatest respect to the Examiner, this was simply not the case at the priority date. While the prior art contains examples of systems having an array of areas of binding agent (for instance a conventional microtitre plate), nowhere is there described or suggested the concept of using less than 0.1V/K moles of binding agent, much less an example of this having been carried out. This is important as the present invention makes it theoretically and practically possible to miniaturize assays capable of giving quantitative results for the first time. This concept is not present in Ekins '031, the closest prior art.

While Ekins '031 concerns reducing the amount of binding agent used under special circumstances to construct assays that are sample-volume independent, this reference does not tackle the wider issue of miniaturization. That requires the disclosure of the present application, e.g. involving the use of microspots and amounts of binding agent less than 0.1V/K (see below).

Thus, it is submitted that the Examiner, while understandably wishing to be thorough in investigating the prior art and advancing counter-arguments to the applicant's position, is nonetheless thereby using hindsight to look back at the invention, rather than looking forward from the priority date. At the priority date, there was no suggestion of trying to reduce the amount of binding agent to these hitherto unthought of levels, and a clear prejudice in the art against doing so. It is even clearer that there is no suggestion that such an assay could be as sensitive as or indeed more sensitive than the prior art assays (see the American Thyroid Association example) which use far larger amounts of binding agent.

VI. EVEN BASED ON THE EXAMINER'S PRACTICAL ANALYSIS, THE PRESENT INVENTION IS NOT FULLY SUGGESTED

The Examiner argues that the reason why people of ordinary skill in the art did not arrive at the present

invention from Ekins '031 was that technical problems prevented them from constructing a sample surface and a device to read the surface, enabling amounts of binding agent less than 0.1V/K to be utilized.

Apart form the Examiner's impermissible use of hindsight and assumptions in this analysis, creating a motivation to reduce the amount of binding agent disclosed in Ekins '031 that never existed, the Examiner himself admits that this would not completely solve the problem. Further, this ignores the prejudice against reducing the amount of binding agent to these levels on the ground that it would be impossible to obtain a sensitive and fast assay by following this strategy.

The reality of the matter is that techniques to immobilize small amounts of binding agent were known, as was the instrumentation to detect fluorescence from small labeled areas. However, even with these techniques being available, those skilled in the art did not conceive that a workable assay system could be developed.

VII. LEABACK REFERENCE ONLY CONCERNS A MEASURING DEVICE AND PROVIDES NO DISCLOSURE CONCERNING OTHER ASPECTS OF ASSAY DESIGN

The Examiner cites Leaback as showing a device capable of monitoring a plurality of small sites simultaneously, presumably to support his argument that the technical means to detect the signal from the microspots of binding agent used in the present application existed in the prior art.

However, as the Examiner admits on page 4, lines 20-22, "Leaback does not completely solve the problem", as there is no disclosure in this reference concerning the amount of binding agent to use, other than it is small, saving on the cost of reagents. Thus, while Leaback claims to be able to provide quantitative measurements of analyte concentration (see column 5, lines 3 and 4), Leaback makes no reference to the amount of binding agent used, the volume of the sample or

the need to immobilize the binding agent as a spot of closely spaced binding agent.

These are all important features of the present invention and Leaback, like Ekins '031, provides no teaching of their importance, much less a teaching to the person of ordinary skill in the art.

VIII. LEABACK DOES NOT DISCLOSE HOW TO IMPROVE ASSAY SENSITIVITY

Leaback attempts to make his assay system more sensitive by compensating for background noise. He does this by effectively subtracting the background reading from the observed signal, see column 5, lines 55-64.

However, as has been known by those skilled in the art for many years, this will not make an assay system any more sensitive. What is important here is the elimination or reduction of background noise (rather than its compensation), i.e. an instrument with a lower background yields greater sensitivity, other things being equal.

Thus, if the background represents about 90% of the total signal, the sensitivity will be low and subtracting the background noise from the signal will not improve that sensitivity. On the other hand, reducing or eliminating the background will enhance sensitivity of the assay.

IX. ADDITIONAL EVIDENCE OF PATENTABILITY

It is noted, in passing, that applications corresponding to this United States application have been filed in many countries, including Europe and Japan, and the objection in light of Ekins '031 has been consistently raised when the applications have reached examination. However, in all cases apart from the United States, arguments similar to those presented above have succeeded in overcoming such objections.

In addition, the scientific community clearly regards Professor Ekins' work as interesting and important. This is evidenced by the fact that Professor Ekins was

recently invited to contribute a chapter to the second edition of the *Principles of Nuclear Medicine*, a prestigious volume that is currently under preparation and is edited by Professor Henry Wagner of the Johns Hopkins University, Baltimore.

In particular, Professor Ekins was asked to discuss in his chapter the issue of sensitivity in relation to assays using binding agent immobilized as microspots. Clearly, even today, the subject matter of the present application is not regarded as a trivial extension of the prior art by those of eminence in the field.

X. CONCLUSION

In view of the foregoing remarks, it is respectfully urged that the rejections set forth in the March 24, 1994 Official Action be withdrawn and that this application be passed to issue, and such action is earnestly solicited.

Respectfully submitted,

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Enclosures: - Declaration of Professor Roger P. Ekins (and attachments)